Annual Report to Air Force Office of Scientific Research (Year 2)

This report covers the work done in the period (4 months) since mid-term report submitted in Spring, 2012.

In the previous report, we have shown that the mechanosensory Böhm's bristles located on the base of the moth antennae are responsible for mediating antennal positioning in the hawk moth *Daphnis nerii*. Ablating these bristles resulted in mispositioning of the antenna, and frequent collisions with the wing during flight. We also found that the sensory neuronal arbors of the bristles spatially overlap with the dendritic arbors of antennal muscle motor neurons. Antennal muscles respond to stimulation of the Böhm's bristles at very short time scales (<10ms) indicating that the connections between them may be monosynaptic. Our data thus suggested that the control of antennal positioning by the Böhm's bristles via a reflex mechanism. We have recently published this paper in the Journal of Experimental Biology.

In the following sections, we outline experiments that show how insect antennal muscles are also influenced by visual inputs. Thus, the neural circuitry underlying the antennal positioning response integrates multi-modal information from both vision and mechanosensation, thus making it a powerful system to study how antennal motor neurons (and neurons in general) integrate and respond to information from multiple modalities. These experiments are conducted at various levels ranging from antennal responses (section 1) and ventral nerve cord recordings (section 2) in moths, to whole animal behavior in bees (section 3)

1. Visual input to the antennal motor system of hawk moths (Anand Krishnan)

Antennal positioning in hawkmoths is one of the first observable behaviours indicating the onset of flight. At rest, hawkmoths tuck their antennae behind the head and under the wing. Upon initiating flight, they rotate their antennae forward and maintain them in a fixed position during flight (Dorsett 1962). Because antennal olfactory and mechanosensory feedback are critically important for various aspects of flight control (Sane et al 2007), correct knowledge of sensor position relative to the body may be important for unambiguous detection of sensory input. Indeed, scapal hair plates in the cockroach have been shown to encode antennal position, thereby enabling tactile detection of objects (Okada and Toh, 2001).

There is, however, a growing body of evidence suggesting that multimodal input may also influence the activity of antennal motor neurons. Antennae in the fruit fly *Drosophila melanogaster* show active and passive responses to moving visual stimuli (Mamiya et al 2011). Neuroanatomical studies on the honeybee *Apis mellifera* have identified motion-sensitive lobular interneurons that arborise in the same region as the antennal sensory afferents (Hertel and Maronde 1987, Maronde 1991). Because visual input plays a role in regulating flight speed (Baird et al 2005, 2010), visual input to the antennae may be relevant to the flying insect.

Methods

We immobilised adult moths (*Daphnis nerii)* by placing them in a sawn-off syringe tube. The preparation was mounted on a pneumatic table under a swivelling dissection microscope (after 1h of recovery), and the left antenna (in which we performed all

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14. ABSTRACT
This is the first annual report detailing work on the mechanosensory Bohm?s bristles located on the base of the moth antennae that are responsible for mediating antennal positioning in the hawk moth Daphnis nerii. The report also outlines experiments to show how insect antennal muscles are also influenced by visual inputs.

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recordings) was inserted into a glass capillary and glued in place at approximately the 5th annulus of the flagellum. We restricted the pedicel-flagellar joint using cyanoacrylate glue to ensure that only the scape was free to move. We inserted a tungsten recording electrode (FHC, Inc. 5 µm diameter, 2MΩ impedance) into the Ms-pp intrinsic muscle (Niehaus and Gewecke 1978), and an indifferent electrode was inserted into the frontal area of the head cuticle. We recorded electromyograms of the intrinsic muscle using a Grass Instruments P55/WPI DAM50 differential AC pre-amplifier, and collected the data using a Data acquisition device (National Instruments/Axon Instruments Digidata 1322A) coupled to either pCLAMP 10.0 or a custom-written labVIEW interface. Visual stimuli were provided using a 1W white light LED. We provided both long 5s pulses of white light, and short 10ms pulses (approximating the impulse response of the system) to both eyes while recording EMGs from the left antenna.

Results

Antennal muscles receive visual input

We recorded electromyograms of the Ms-pp antennal intrinsic muscle while providing static visual stimuli (a 5s pulse of white light). The intrinsic muscles displayed phasic excitatory responses to pulses of light provided to both the ipsilateral and contralateral eyes.

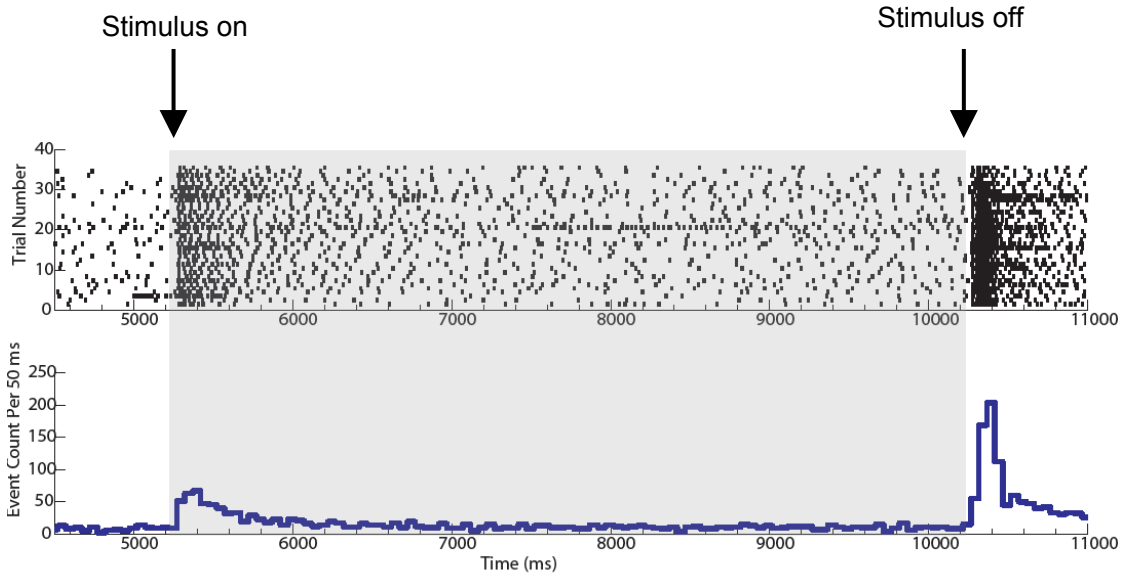

Figure 1: Intrinsic muscle responses to visual stimulation.

Antennal muscle responses to visual stimulation are 3-4 times slower than to mechanosensory stimulation

To obtain the response latencies of the antennal muscles to visual stimuli, we stimulated the eyes with a 10ms short pulse of white light, and used the muscle responses to calculate the latency to significant firing shift, or the point where the firing rate crossed 5 standard deviations from the mean (as in Krishnan et al, 2012). Visually-mediated responses of the antennal muscles occur at time scales 3-4 times slower than the responses to mechanosensory stimulation, suggesting that visual input to the antennae is involved in slower, long-latency aspects of antennal positioning behaviour.

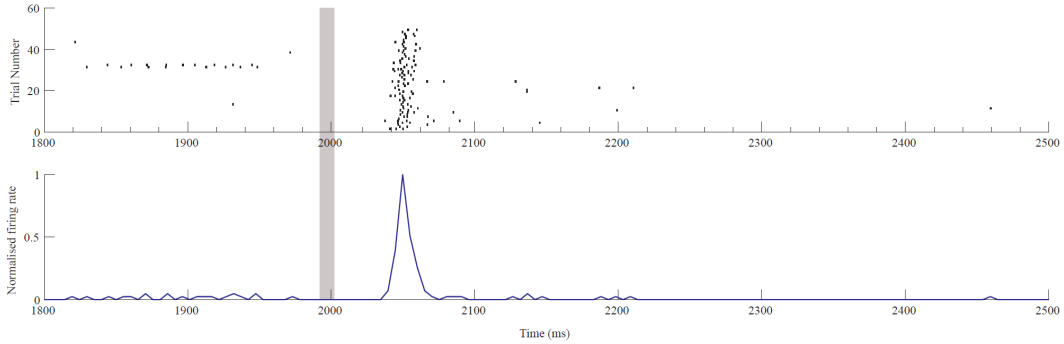

Figure 2: Intrinsic muscle responses to a 10ms pulse of white light.

2. Neural mechanisms of flight control in moths (Umesh Mohan, Sunil Prabhakar)

In the previous report, we had described the work on developing mechanical stimulus apparatus. Here we present work on developing a visual stimulus apparatus and intracellular recording in the ventral nerve cord of moth.

Visual stimulus delivery apparatus:

To determine the response of moth to various visual stimuli, we needed a mechanism to generate controlled visual stimulus. The requirement, as determined from an extensive electroretinogram study taken in lab, was an apparatus which can produce visual stimuli with an angular resolution of 3°at the moth's eye with flicker frequency greater than 200 Hz. After looking at various possibilities, we decided to build a circular green LED strip (Block diagram : Fig 3, Schematic : Fig 4, Photo of the setup : Fig 5). This year we intend to extend this into a 2 dimensional LED arena to give more complex visual stimuli to the moth.

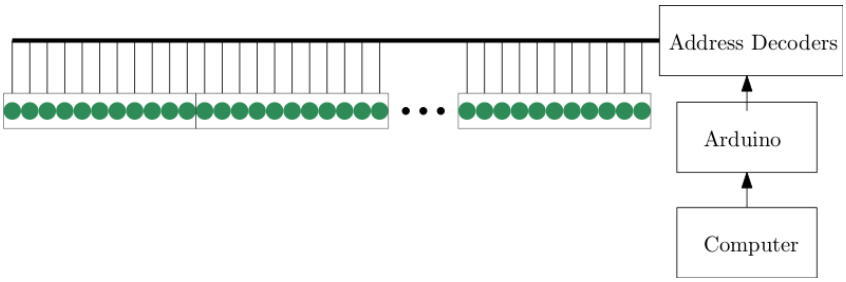

Figure 3: LED strip hardware

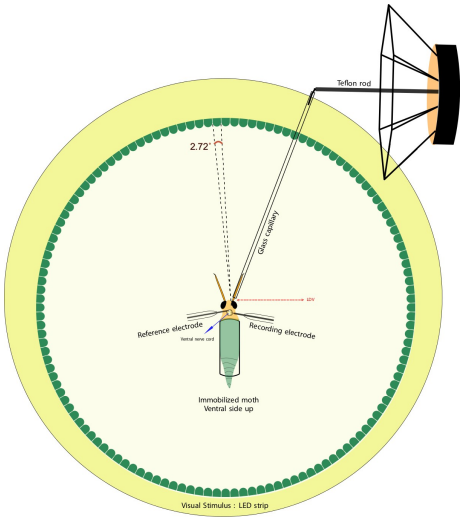

Figure 4: Schematic of the intracellular recording setup and sensory stimulation apparatus

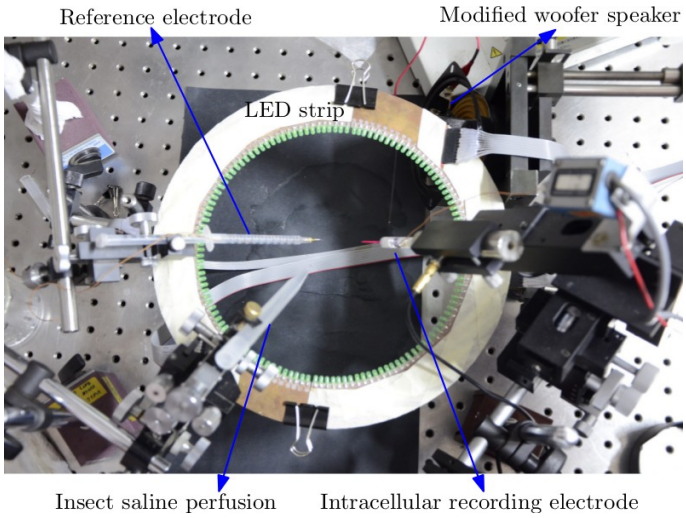

Figure 5: Visual and Mechanical stimulus in the intracellular recording setup

Once the mechanical and visual stimulus generation mechanism were developed and characterized, we setup a system to record from the ventral nerve cord of the moth (Schematic : Fig 4, Photo of the setup : Fig 5). We identified neurons in the ventral nerve cord that responded to mechanical stimulus and visual stimulus.

Mechanical stimulus: We gave step stimulus (1° deflection in 10 ms) to the moth's antenna. Response of neuron in the ventral nerve cord to this mechanical stimulus is shown in Fig 6.

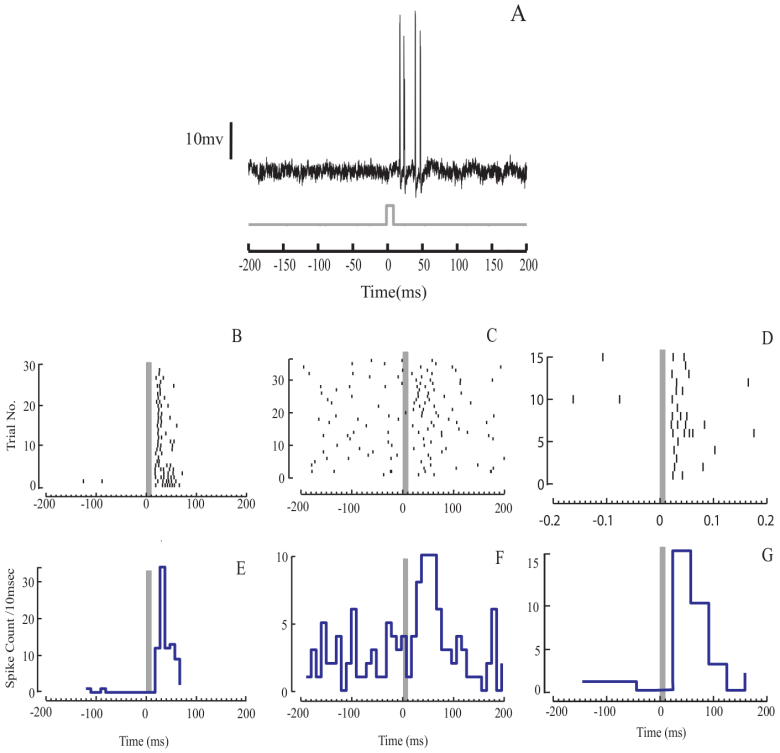

Figure 6: Descending interneurons respond to mechanical stimulation of the antenna

A. Representative plot of a unit trial recorded in response to the mechanical stimulus (grey pulse). The black trace is an example of spikes recorded in response to the stimulus. For a neuron, spikes occurring in a time window of 200ms before and after stimulus onset are included to compare responses across all trials. 0 on the x-axis indicates the time of stimulus onset.

B, C & D. Peri-stimulus time raster plots constructed for different neurons. Each spike is marked by a line on the plot whose position is function of trial number and the time of its occurrence. The grey bar represents the duration of mechanical stimulus

E, F & G. Peri-stimulus time histograms for raster plots shown in B, C & D respectively. The analysis time window (400ms) is divided into 40 bins each of width 10ms. Spikes falling into the respective bins are counted to plot a cumulative peri-stimulus time histogram

Visual stimulus: We gave optical stimulus in the form of two spots of light (one in each half-section of LED arc) moving from anterior to the posterior of the animal. The rate of optic flow translation was kept constant. Response of neuron in the ventral nerve cord to this visual stimulus is shown in Fig 7.

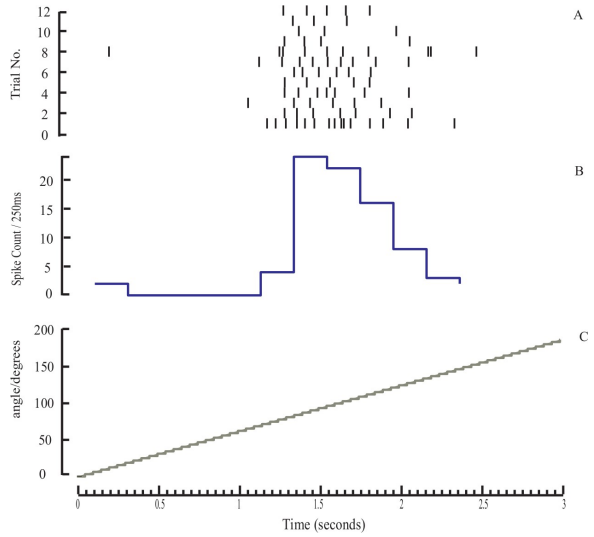

Figure 7: Optic flow stimuli elicit spiking responses in the descending interneurons

A. Raster representation of spikes occurring in each trial of the visual stimulation.

B. A cumulative histogram obtained by counting the number of spikes occurring in each bin of width 250ms across all trials.

C. Representative plot of the optic flow stimulus. Unit shift of the angle is plotted against time to obtain a ramp which indicates an angle shift from 0-180° which corresponds to optical motion of the spotlight from anterior(0°) to posterior(180°) of the animal.

At the end of recording responses of the neuron in ventral nerve cord to visual and mechanical stimulus, we injected a dye (Alexa Fluor 568) *via* the recording electrode into the neuron. We then sacrificed and dissected the moth to isolate its nervous system. We then imaged this isolated nervous system to detect the arborization regions of the neuron which was recorded from. A sketch of the neuron recovered through this imaging method is shown in Fig 8.

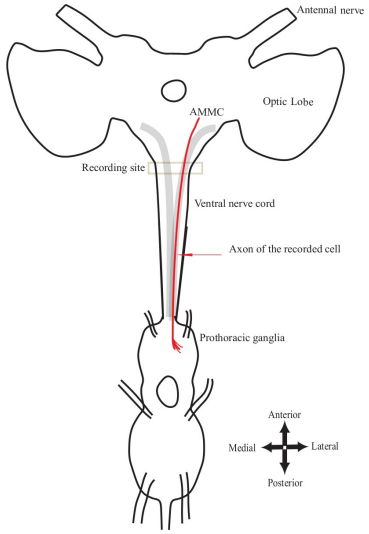

Figure 8: Axonal projections of an antennal mechanoresponsive descending interneuron. The diagram is a cartoon representation of the Daphnis nerii nervous system. The grey chords indicate the location of the two neck connective bundles. The red trace is a sketch of the dye fill recovered of a descending interneuron found to be responsive for antennal mechanosensory stimulations. The recording site is marked by a grey rectangular window.

3. Antennal responses to airflow in flying honey bees: (Taruni Roy)

Behavioral characterization of antennal positioning response in honey bees

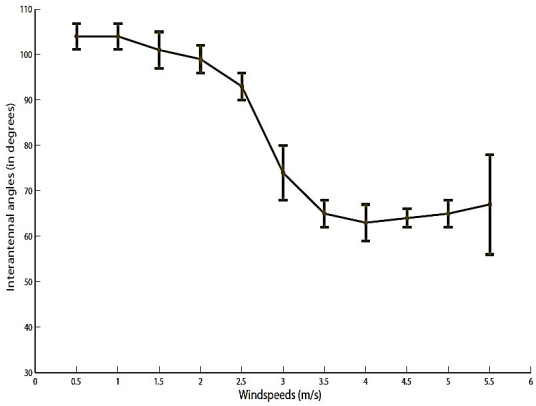

Figure 9: Plot of wind speeds vs Inter antennal angles for a tethered bee in a wind tunnel. The wind speeds decreased linearly from 5.5 m/s to 0 m/s in a single flight bout of the same bee whose photographs are shown.

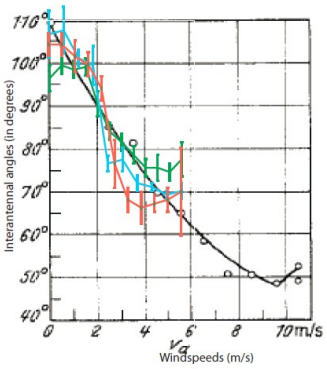

Figure 10: Our data (coloured) from 3 bees superimposed on an older set of measurements by Heran (black).

Our experiments demonstrate that the antennae in tethered honey bees in the wind tunnel respond to wind flow in a sigmoidal fashion. In contrast to the reported linear decline (Heran, 1955), the antennae appear to have a saturated response at low windspeeds and very high windspeeds. This is interesting because the low windspeeds correspond to the reported airspeed of the bee flying in lab conditions (~0.5 m/s) and the high windspeeds correspond to the airspeeds of the bee flying in natural open environments (~ 6m/s Wenner 1963). Also, even if the set point of each bee is different, the nature of this behaviour is consistent between individuals with an almost similar point of change from saturated response to monotonicity (~2/2.5 m/s) and from monotonic decline to saturation (3/3.5 m/s). So, the antennae do not respond by changing the interantennal angles at the reported airspeeds of the bee both in the lab and in open natural environment but they can detect airflow experienced by the flying animal.